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Dormant Spores Receive an Unexpected Wake-up Call

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Germination of spores of *Bacillus* bacteria can be triggered by nutrients acting through receptors on the spore's inner membrane. Shah et al. (2008) now report that cell wall peptidoglycan fragments can also trigger spore germination by binding to an inner membrane-bound protein kinase.

In *Bacillus* bacteria, the process of sporulation is triggered by nutrient starvation, and the resulting spores are metabolically dormant and extremely resistant to a variety of stress factors (Setlow, 2006). As a consequence, spores can survive for long periods of time, certainly for hundreds of years if not more. However, even though these spores are dormant, they are continually monitoring their environment, and if conditions favorable for cell growth return, the spores can rapidly break dormancy and "return to life" (Setlow, 2003). In the current model for spore germination, specific nutrient molecules bind to their cognate germinant receptors located in the inner spore membrane (Figure 1A). This leads to the release of small molecules from the spore core, most notably the huge depot of dipicolinic acid chelated 1:1 with divalent cations, largely Ca^{2+} (Ca-DPA), and replacement of these small molecules by water. In the dormant spore core, water is maintained at an extremely low level, likely to prevent enzymatic activity. The release of Ca-DPA activates enzymes that can degrade the spore's peptidoglycan cortex restricting the swelling of the spore core. Hydrolysis of the cortex leads to the swelling of the spore core and further water uptake (Figure 1B). The water content is then high enough to allow enzyme action, metabolism, and macromolecular synthesis, with vegetative growth following soon thereafter.

In this issue, Shah et al. (2008) report evidence for an alternative and very different mechanism for initiating germination, based largely on work with the model organism and spore former *Bacillus subtilis*, a species of Gram-positive bacteria. They find that germination of *B. subtilis* spores, as well as those of several other *Bacillus* species, can be triggered by extremely low concentrations (≤1 pg/ml) of muropeptides, which are produced by degradation of the peptidoglycan that comprises the cell wall of most bacteria. Such degradation is a normal feature of bacterial cell growth. In growing Gram-negative bacteria, muropeptides are not generally released in significant amounts due in large part to efficient recycling, whereas Gram-positive bacteria recycle muropeptides poorly (Doyle et al., 1988; Mauck et al., 1971). As a consequence, high levels of muropeptides in the environment could indicate abundant growth of Gram-positive bacteria and thus provide a logical environmental signal that could initiate spore germination. The muropeptide signal leading to *B. subtilis* spore germination is not just from any muropeptide but only from those that have the amino acid *meso*-diaminopimelate (Dpm) at position three of the stem peptide linked to the muropeptide's oligosaccharide backbone. Dpm is the amino acid found in this position in peptidoglycan of growing *B. subtilis* cells. In contrast, the peptidoglycan from at least one other *Bacillus* species, *B. sphaericus*, and other Gram-positive bacteria such as *Staphylococcus aureus* contain L-lysine in the third position of the stem peptide (Hungerer and Tipper, 1969; Schleifer and Kandler, 1972), and muropeptides from this type of peptidoglycan are ineffective at eliciting *B. subtilis* spore germination.

Notably, the triggering of spore germination by the appropriate muropeptides occurs in the absence of all nutrient germinant receptors, suggesting that muropeptides do not trigger germination through the nutrient germinant receptors. As a consequence, Shah et al. (2008) sought to identify bacterial

proteins that bind peptidoglycan that could serve as the signal transducer. The authors focused on membrane proteins given that the nutrient germinant receptors are located in the spore's inner membrane where initial germination signaling takes place. This led to the identification of a member of a serine/threonine protein kinase family, PrkC, which contains multiple PASTA (*p*enicillin *a*nd *s*erine/*t*hreonine kinase *a*ssociated) repeats. PASTA repeats have been proposed to bind peptidoglycan, in particular the stem peptide (Jones and Dyson, 2006; Yeats et al., 2002), and are typically found on the extracellular side of membranes. The environmental muropeptides of the size capable of triggering germination should readily pass through the spore's outer layers of coat, outer membrane, cortex, and germ cell wall to the outer surface of the inner membrane (Setlow, 2006) (Figure 1A). Strikingly, loss of PrkC or mutation of a residue that is likely essential for PrkC catalysis abolishes muropeptide germination of *B. subtilis* spores, as well as spores of the pathogenic species *B. anthracis*. PrkC from *B. subtilis* is also located in the spore's inner membrane and appears to phosphorylate at least one protein, translation elongation factor-G, in germinated spores. In addition, the PASTA repeats of *B. subtilis* PrkC bind peptidoglycan with Dpm in the third position of the stem peptide but not peptidoglycan in which L-lysine replaces Dpm. In contrast, PrkC from *S. aureus* binds *S. aureus* peptidoglycan that contains L-lysine in the stem peptide, and *B. subtilis* spores engineered to contain only *S. aureus* PrkC germinate well with muropeptides from *S. aureus* peptidoglycan. As further evidence that PrkC is involved in spore germination due to muropeptides, and not in some aspect of spore formation, bryostatin, a known activator of serine/threonine kinases of the PrkC family, is an efficient trigger of germination in wild-type spores but not in spores lacking PrkC. In addition staurosporine, an inhibitor of serine/threonine kinases similar to PrkC, inhibits muropeptide germination of *B. subtilis* spores at 10 pM but is ineffective at inhibiting spore germination via the nutrient germinant receptors.

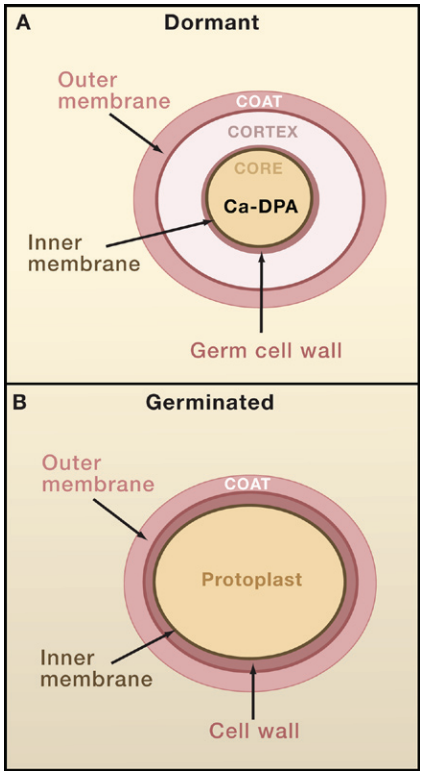

Figure 1. Dormant and Germinated *Bacillus* Spores

Schematic structure of a *Bacillus subtilis* spore when dormant (A) and fully germinated (B). The relative sizes of the various spore layers are approximate and no outermost exosporium is shown, as this layer is likely absent from *B. subtilis* spores. The coat, outer membrane, and cortex of dormant spores are not an effective permeability barrier to passage of small molecules (Setlow, 2006), such as muropeptides, which are produced by degradation of the peptidoglycan that comprises the cell wall. Shah et al. (2008) now show that muropeptides serve as a trigger for germination. Germination is characterized by the release of a depot of dipicolinic acid chelated 1:1 with Ca^{2+} (Ca-DPA) from the core of the dormant spore. The core, now lacking Ca-DPA and swollen following cortex degradation, becomes the protoplast of the germinated spore. Although the germ cell wall in the dormant spore is composed of peptidoglycan, it has a different structure than peptidoglycan of the cortex. The germ cell wall is not degraded in germination and provides the foundation for the new cell wall; ultimately the spore coat and outer membrane are shed as the germinated spore transitions to a growing cell.

The identification of this muropeptide germination pathway opens up a whole new way of looking at spore germination and suggests that not only are the nutrient germinant receptors and the enzymes that degrade the spore cortex redundant, but so are the pathways that initiate the germination process. However, a number of important questions remain. (1) Do muropeptides released from germinating spores trigger spore germination? As noted above, cortex peptidoglycan is degraded during germination, and the resultant muropeptides are largely released into the medium. Triggering of germination by these released muropeptides might be the reason that in some cases spore germination efficiency increases at higher spore concentrations. However, if spore germination is too responsive to compounds released from germinating spores, one spore that inappropriately germinates might lead all of its brothers and sisters to germinate and then die, much like lemmings leaping into the sea. (2) Do spores have mechanisms to guard against inappropriate germination if levels of muropeptides are high because of the death and lysis of growing cells, perhaps due to the presence of a toxin? (3) It is known that some spores germinate extremely poorly with nutrients, whereas others respond even to low nutrient levels. Perhaps this heterogeneity is important in ensuring that not all spores in a population germinate simultaneously and perhaps inappropriately. Is this same heterogeneity seen with muropeptide germination? (4) Is there any interaction or synergy between the nutrient and muropeptide germination pathways to allow a spore to integrate signals from these two types of germinants? Signal integration does take place in the response to nutrients, although the mechanism of this integration is not known. (5) How does muropeptide binding to PrkC lead to activation of downstream germination events, in particular release of Ca-DPA? (6) How does PrkC's catalytic activity trigger germination? Dormant spores have little if any ATP, and significant ATP levels are not generated until after germination has been triggered (Setlow, 2003). In addition, it appears likely that enzymes are not active in the spore core. Thus it is not clear how PrkC could be catalytically active given the environment of the dormant spore. Although there are many questions remaining, the identification of the muropeptide germination pathway has certainly added a new dimension to our understanding of bacterial spore germination and given us much to think about.

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A Protein-Only RNase P in Human Mitochondria

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In bacteria, archaea, and the eukaryote nucleus, the endonuclease ribonuclease P (RNase P) is composed of a catalytic RNA that is assisted by protein subunits. Holzmann et al. (2008) now provide evidence that the human mitochondrial RNase P is an entirely protein-based enzyme.

Ribonuclease P (RNase P) is responsible for the 5′ maturation of precursor transfer RNAs (pre-tRNAs). The catalytic RNA subunit of bacterial RNase P was one of the first examples of RNA-based catalysis. All forms of RNase P characterized to date have a fundamentally similar RNA subunit that retains catalytic activity. This catalytic RNA is widely presumed to be a remnant of the hypothesized "RNA world" in which RNA is thought to have been the original functional macromolecule preceding the evolution of protein. Although the nature of the RNase P catalytic RNA seems evolutionarily conserved, the number of proteins associated with the RNA actually increases with the complexity of the organism—ranging from one in bacteria to at least four in archaea and to at least nine in eukaryotes (Figure 1)

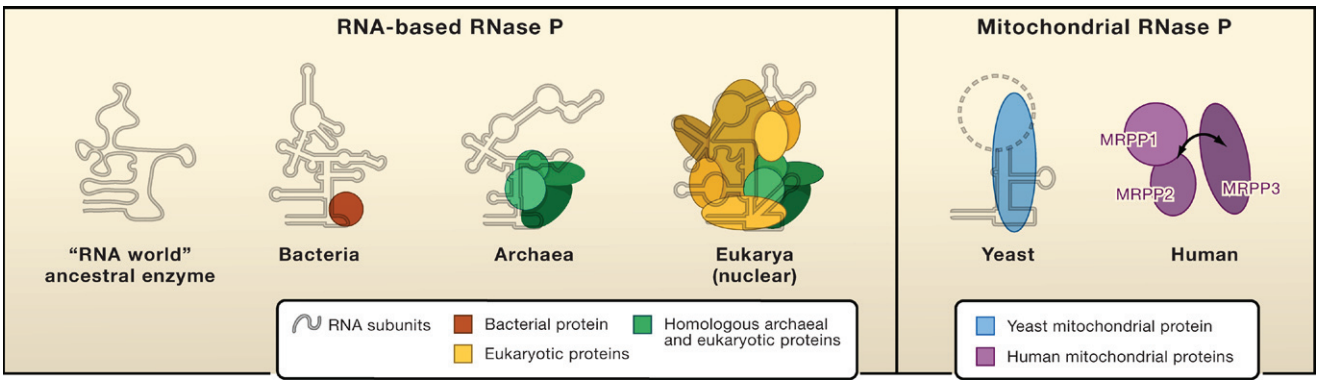

Figure 1. The Evolution of RNase P

(Left) The compositions of characterized RNA-based RNase P enzymes from bacteria, archaea, and eukarya show an increase in protein content with increased complexity of the organism. The sites of interaction between RNase P subunits are not known in most cases and are represented schematically. The structure of the proposed ancestral RNA-only RNase P is not known and is assumed to have the critical structural elements conserved in all forms of RNase P RNA.

(Right) The composition of the fully characterized mitochondrial RNase P is shown for yeast (*S. cerevisiae*) and human (*H. sapiens*). Human mtRNase P is composed only of proteins (mitochondrial RNase P proteins 1, 2, 3) (Holzmann et al., 2008). The third subunit of the human mtRNase P (MRPP3) binds to the two-protein subcomplex weakly and may associate dynamically (arrow). Although key structural elements of the RNA subunit are preserved in various yeast mtRNase P enzymes (solid line), the entire RNA structure is not well defined (dashed line).